Cytokeratin 18 Fragment Levels as a Noninvasive Biomarker for Nonalcoholic Steatohepatitis in Bariatric Surgery Patients

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Background & Aims: **Nonalcoholic fatty liver disease (NAFLD) is extremely common among morbidly obese patients. We assessed the usefulness of plasma caspase–generated cytokeratin 18 (CK-18) fragments as a novel marker for NAFLD in a bariatric cohort.** ***Methods:*** **The cohort consisted of 99 consecutive patients who underwent liver biopsy at the time of bariatric surgery. CK-18 levels were measured by using an enzyme-linked immunosorbent assay before and 6 months after surgery. Patients were subdivided into 4 histologic groups: not NAFLD (normal liver biopsy), nonalcoholic fatty liver (NAFL), borderline diagnosis, and definitive nonalcoholic steatohepatitis (NASH).** ***Results:*** **CK-18 levels were significantly higher in subjects with NASH compared with those with not NAFLD, NAFL, or borderline diagnosis (median [25th quartile, 75th quartile], 389 U/L [275, 839] vs 196 U/L [158, 245], vs 217 U/L [154, 228], or vs 200 U/L [176, 274], respectively; $P < .0001$). CK-18 levels were significantly higher in subjects with moderate to severe fibrosis versus those with no or mild fibrosis (334.5 U/L [240.5, 896] vs 207 U/L [175, 275], respectively; $P = .007$). A significant decrease in CK-18 levels was observed in most patients 6 months postoperatively. The area under the receiver operating characteristic curve for NASH diagnosis was estimated to be 0.88 (95% confidence interval, 0.77–0.99). The values with the best combination of sensitivity and specificity were 252 U/L (sensitivity, 82%; specificity, 77%) and 275 U/L (sensitivity, 77%; specificity, 100%).** ***Conclusions:*** **These results support the potential utility of this test for diagnosis and staging of NAFLD before bariatric surgery.**

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States and occurs mainly in overweight or obese individuals.[1,2] It is extremely common among patients undergoing bariatric surgery.[3,4] NAFLD encompasses a wide spectrum of conditions ranging from nonalcoholic fatty liver (NAFL) or steatosis to nonalcoholic steatohepatitis (NASH) to cirrhosis.[5] NASH is a potentially serious condition because as many as 25% of patients might progress to cirrhosis and experience its complications.[6,7] A liver biopsy remains the only reliable way to differentiate steatosis from NASH and to determine the stage and grade of the disease.[8]

Morbidly obese patients undergoing bariatric surgery are a group at particular risk for NAFLD and for development of the more serious forms of this condition.[9,10] Development of reliable noninvasive biomarkers to diagnose and determine disease severity before bariatric surgery and to monitor disease status postoperatively would be of significant clinical utility.

Increased hepatocyte death by apoptosis might play an important role in liver injury and disease progression in NAFLD.[11] During the process of apoptosis, effector caspases (mainly caspase 3) are activated and cleave a number of substrates inside the cell including cytokeratin 18 (CK-18), the major intermediate filament protein in the liver, resulting in the characteristic morphologic changes of apoptosis.[11] Previously, we demonstrated that the plasma concentration of caspase-generated CK-18 fragments accurately differentiates NASH from NAFL and predicts stage of fibrosis in patients with NAFLD.[12,13] The aim of this study was to assess the utility of this novel biomarker in determining NASH, assessing disease severity, and monitoring disease status after bariatric surgery in morbidly obese patients.

Patients and Methods

Patient Characteristics

The study was approved by the Cleveland Clinic Institutional Review Board. Our cohort consisted of 99 consecutive patients who underwent liver biopsy at the time of bariatric surgery as part of a standard clinical procedure. The diagnosis of NAFLD was based on liver biopsy features as assessed by an experienced hepatopathologist (L.Y.). Patients were subdivided into 4 histologic groups: not NAFLD (normal liver biopsy), NAFL, borderline diagnosis, and definitive NASH. The NAFLD National Institute of Diabetes and Digestive and Kidney Diseases activity score[14] was applied to each patient. Demographic, clinical, and laboratory data were obtained from clinic visits (2–4 weeks) before surgery. The absence of current excessive alcohol use was defined by an average daily consumption of alcohol of <20 g/day for men and <10 g/day for women. Prevalence of diabetes, hypertension, and hyperlipidemia was assessed by review of past medical history. Prevalence of diabetes was based on medical history and/or fasting plasma glucose of 126 mg/dL or greater.

Abbreviations used in this paper: BMI, body mass index; CI, confidence interval; CK, cytokeratin; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ROC, receiver operating characteristic.

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Liver Histology

The histologic diagnosis of NAFLD was established by the study pathologist according to her expertise and following the NAS in a blinded manner regarding the CK-18 fragment measurements and patient's clinical and laboratory data.[14] In this scoring system, the degree of steatosis, liver injury, and inflammatory activity is measured by using an 8-point scale (steatosis, 0–3; lobular inflammation, 0–3; ballooning degeneration of hepatocytes, 0–2). The NAS is the unweighted sum of steatosis, lobular inflammation, and hepatocellular ballooning scores. The stage of fibrosis was similarly measured by using a 6-point scale (1a, b = mild (1a)/moderate (1b) zone 3 perisinusoidal fibrosis; 1c = portal fibrosis only; 2 = zone 3 and portal/periportal fibrosis; 3 = bridging fibrosis; 4 = cirrhosis).

Measurement of Caspase-Generated Cytokeratin-18 Fragments in the Blood

CK-18 levels were measured in 86 patients who had plasma available within 1 week before surgery by using a sandwich immuno enzyme-linked immunosorbent assay (ELISA) specific for CK-18 fragments. In addition, CK-18 levels were measured 6 months after bariatric surgery in those patients with available plasma (n = 34). All samples were initially processed to plasma and stored frozen at −80°C. The plasma was subsequently used for quantitative measurement of the apoptosis-associated neoepitope in the C-terminal domain of CK-18 by the M30-Apoptosense ELISA kit (PEVIVA; Alexis, Grünwald, Germany). All assays were performed in duplicate, and the absorbance was determined by using a microplate reader (Molecular Devices M2, Sunnyvale, CA).

Statistical Analysis

Descriptive statistics were computed for all variables. These included means and standard deviations or medians, as well as 25th and 75th percentiles for continuous factors. For categorical variables, frequencies and percentages were estimated. Kruskal-Wallis and Dunn tests were used to assess whether CK-18 levels were significantly different between the 3 subject groups. In addition, Wilcoxon rank sum tests were used to compare CK-18 levels between subjects with moderate to severe fibrosis and those with mild fibrosis. Spearman correlation coefficients were used to assess associations between CK-18 levels and histologic characteristics. Logistic regression analysis was used to assess the association between plasma levels of CK-18 fragments and the likelihood of having definitive NASH as opposed to simple steatosis. To predict the presence of NASH with optimal sensitivity and specificity, receiver operating characteristic (ROC) curve analysis was used to estimate potential cut-off values of plasma CK-18 fragments. The same was done to assess the utility of CK-18 levels in the prediction of fibrosis. A P value of .05 was considered statistically significant. SAS version 9.1 software (SAS Institute, Cary, NC) and R 2.0.1 software (The R Foundation for Statistical Computing) were used to perform all analyses.

Results

Characteristics of the Patient Population

The main clinical and laboratory characteristics of the patients are described in Table 1, and the histologic characteristics of the liver biopsies are summarized in Table 2. The patients' age (median, 51 years), gender (68% female), and body mass index (BMI) (median, 48 kg/m^2) did not statistically differ among the 4 histologic groups. There was no difference in the prevalence of diabetes, hypertension, or hyperlipidemia among the groups. Serum AST and ALT were within the normal range in most patients, although subjects with NASH tended to have significantly higher AST and ALT levels than both subjects without NAFLD and those with NAFL. In addition, borderline subjects had higher ALT levels than those without NAFLD.

Table 1. Demographic and Clinical Characteristics of Subjects Who Underwent Bariatric Surgery

Factor	All (N = 86)	Not NAFLD (N = 21)	NAFL (N = 13)	Borderline (N = 30)	NASH (N = 22)	P value
Age (*y*)	51.0 (41.0, 56.0)	51.0 (41.0, 57.0)	46.0 (42.0, 51.0)	52.5 (42.0, 56.0)	50.0 (40.0, 56.0)	.68
BMI (*kg/m^2*)	48.0 (43.0, 54.0)	48.0 (46.0, 54.0)	48.0 (45.0, 51.0)	48.0 (42.0, 54.0)	47.5 (42.0, 55.0)	.93
AST (*U/L*)	23.0 (18.0, 29.0)	19.0 (16.0, 24.0)	20.0 (17.0, 23.0)	23.5 (19.0, 29.0)	28.5 (23.5, 50.5)	.01
ALT (*U/L*)	21.5 (16.0, 33.0)	17.0 (11.0, 19.0)	17.0 (15.0, 20.0)	26.0 (19.0, 34.0)	33.5 (22.0, 61.5)	.001
Gender						.98
Male (%)	18 (20.9)	4 (19.1)	3 (23.1)	7 (23.3)	4 (18.2)	
Female (%)	68 (79.1)	17 (81.0)	10 (76.9)	23 (76.7)	18 (81.8)	
Ethnicity						.04
White (%)	70 (81.4)	15 (71.4)	8 (61.5)	26 (86.7)	21 (95.5)	
Other (%)	16 (18.6)	6 (28.6)	5 (38.5)	4 (13.3)	1 (4.6)	
Diabetes						.92
No (%)	51 (59.3)	12 (57.1)	8 (61.5)	19 (63.3)	12 (54.6)	
Yes (%)	35 (40.7)	9 (42.9)	5 (38.5)	11 (36.7)	10 (45.5)	
Hypertension						.7
No (%)	28 (32.6)	9 (42.9)	4 (30.8)	9 (30.0)	6 (27.3)	
Yes (%)	58 (67.4)	12 (57.1)	9 (69.2)	21 (70.0)	16 (72.7)	
Dyslipidemia						.96
No (%)	37 (43.0)	9 (42.9)	5 (38.5)	14 (46.7)	9 (40.9)	
Yes (%)	49 (57.0)	12 (57.1)	8 (61.5)	16 (53.3)	13 (59.1)	

NOTE. Statistics include number (%) or median (25th and 75th percentiles).